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New Validated RP-HPLC Method for Cisplatin and Topotecan in API and Vaccine Form and its Stress Studies

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ABSTRACT

We have developed a completely unique and reliable HPLC technique for simultaneous quantification of Cisplatin and Topotecan. A chromatographic detachment was attained on a XDB C_{18} column (150x4.6mm, 3.5 μ) using isocratic elution with a buffer containing buffer and acetonitrile with the proportion of 60:40 as a movable phase with a flow of 1 mL/min at room temperature and UV detection was carried out at 262 nm. Dissolve 1mL of triethylamine in 1 lt of HPLC grade water and filter through 0.45 μ filter paper. This solution was used as a buffer. 10 min run time was used to separate Cisplatin and Topotecan. The analysis was achieved within 15 min over honest linearity within the concentration range from 5-75 μg/mL of Cisplatin and 2-30 μg/mL of Topotecan. By injecting the standard six times, system suitability parameters were studied and the outcomes were under the acceptable limit. Precision and recovery study results were found to be within a suitable limit. By using the above technique, the assay of the marketed formulation was performed and found to be within the limit. Degradation studies were carried out on Cisplatin and Topotecan, with a purity threshold greater than the purity angle in all conditions and within the acceptable range. The above-mentioned technique was validated according to ICH guidelines.

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INTRODUCTION

Cisplatin is a chemotherapy medication (Alfarouk *et al.*, 2015; Wagner *et al.*, 2017) which is used in the treatment of a number of cancer sicknesses. These include testicular cancer (Hayes-Lattin *et al.*, 2009), cancer of the ovaries (Maoz *et al.*, 2020), cancer of the cervix (Bosch and de Sanjose, 2007), cancer of the breast (Boyd *et al.*, 2007), cancer of the bladder (Suriano *et al.*, 2013), cancer of the head and neck (Vigneswaran and Williams, 2014), cancer of the oesophagus (Lao-Sirieix *et al.*, 2010), cancer of the lungs (Van Schil *et al.*, 2018), cancer of the mesothelioma (Kondola *et al.*, 2016), cancer of the brain (Bondy *et al.*, 2008) and neuroblastoma (Yu *et al.*, 2010). It is administered into a vein by injection. Bone marrow suppression (Hirbe *et al.*, 2007), hearing problems, kidney problems and vomiting (Oun *et al.*, 2018) are common side effects. Other serious adverse effects include numbness, difficulty in walking, allergic reactions, problems with electrolytes (Alfarouk *et al.*, 2020) and heart disease (Chou *et al.*, 2016). Using this medicine dur-

ing pregnancy can result in harming the baby in the womb. Cisplatin is in the anti-neoplastic platinum-based (Falcetta *et al.*, 2016) family of medications. By binding to DNA and inhibiting its replication, it works in part.

Topotecan (trade name Hycamtin) is a chemotherapeutic agent who is an inhibitor of topoisomerase (Mitscher, 2005). It is a synthetic, water-soluble analogue of camptothecin (Chung *et al.*, 2006), a natural chemical compound. It is used to treat ovarian cancer, lung cancer and other types of cancer in the form of the hydrochloride salt. Experiments with neuroblastoma, brainstem glioma, Ewing's sarcoma (Myatt and Burchill, 2008) and Angelman's syndrome (Galassi *et al.*, 2016) have been underway since 2016. Topotecan is also utilized to handle non-small cell lung cancer, colorectal cancer (Domingo *et al.*, 2016), breast cancer, non-Hodgkin lymphoma, Endometrial cancer (Nicolaije *et al.*, 2013) and Oligodendroglioma (Haglof *et al.*, 2006). Figure 1 represents the chemical representation of Cisplatin and Topotecan.

Experiment

Chemicals and Reagents

Acetonitrile and Orthophosphoric acid, triethylamine, water (HPLC grade) were acquired from Merck (India) Ltd, Worli, Mumbai, India. The reference standards of Cisplatin and Topotecan APIs were taken from Spectrum Pharma Research Solutions Pvt. Ltd., Hyderabad.

Equipment

An HPLC system (Waters alliance e2695 model) consisting of a quaternary pump, PDA detector-2998 was used. Data processing was performed with Empower 2.0 software.

Chromatographic Conditions

A chromatographic detachment was achieved in isocratic mode at room temperature using XDB column of C_{18} (150x4.6 mm, 3.5 μ). A mixture of acetonitrile and 0.1 percent triethylamine (TEA) in 40:60 v/v at a stream of 1 mL/min was used as a movable phase. The injection volume was 10 μl and the run time was 10.0 min.

Preparation of Buffer

1 mL of triethylamine is dissolved in 1 litre of HPLC grade water and filtered through 0.45 μ filter paper.

Diluent

The movable phase was used as diluent.

Standard Preparation

Carefully weigh and transfer 50 mg of cisplatin and 20 mg of topotecan in a volumetric flask of capacity 100 mL and add the app. 70 mL of diluents, sonicated to melt it for 30 min. and add diluents up to mark. Further, dilute 5 mL of the above solution to 50 mL with diluents.

Sample Preparation

Carefully weigh and transfer weight similar to 50 mg of cisplatin injection powder and 20 mg of topotecan injection powder in a flask of 100 mL and add 70 mL of diluent. Sonicated to melt and dilute up to the mark with diluent. Take 5 mL of the above solution and dilute it to 50 mL and filtered through a 0.45 μ nylon syringe filter.

Method Validation

System Suitability

In order to confirm the system performance, system suitability parameters were measured. The parameters, including USP plate count, USP tailing and %RSD, were calculated and found to be within the limit.

Specificity

Specificity is the capacity to evaluate the analyte unambiguously the analyte in the presence of other constituents that may be expected to be combined in the sample and standard solution. It is checked by examining the chromatograms of blank samples and samples spiked with cisplatin and topotecan.

Accuracy

Accuracy is the closeness of the test findings gained by the technique to the real value. It is assessed by the recovery studies will be evaluated at three different concentration levels. In each level, a minimum of three injections was administered at each level and quantity of the drug present, percentage recovery and the associated standard deviations were calculated.

Precision

The precision of the analytical technique is the degree of agreement among independent test results. It was studied by analysis of multiple sampling of a homogeneous sample. The precision of the present method was assessed in terms of repeatability, intra-day and inter-day variations. It was checked by analyzing the samples at different time intervals of the same day as well as on different days.

Linearity

The linearity of an analytical method is its ability to obtain results in a defined range directly proportional to the concentration of the analyte in the sample within a definite range. The six series of standard solutions were selected for assessing linearity

Table 1: Optimized Chromatographic Conditions

Parameter	Proposed method
Stationary Phase	XDB C18 (150x4.6 mm, 3.5 μ)
Mobile Phase	0.1% Triethylamine: Acetonitrile (60:40)
Injection Volume	10 μl
Flow Rate	1.0 mL/min
Column Temperature	25°C
Wave Length	262 nm
Run Time	10.0 min
Retention time of Cisplatin	4.358 min
Retention time of Topotecan	7.744 min

Table 2: Results of System Suitability

Parameter	Cisplatin	Topotecan
Theoretical plate count	6261	11234
Tailing factor	1.06	1.14
Resolution	-	13.21
Retention time	4.358	7.744

Table 3: Results of Linearity

S. No	Cisplatin		Topotecan	
	Concentration (μg/mL)	Area	Concentration (μg/mL)	Area
1	5	345271	2	182053
2	12.5	932015	5	615013
3	25	1802501	10	1152468
4	50	3520164	20	2154785
5	62.5	4265021	25	2785436
6	75	5159206	30	3205684

Table 4: Results of Method Precision

S. No.	Area of Cisplatin	Area of Topotecan
1	3562417	2145012
2	3501269	2150234
3	3542815	2145025
4	3546251	2145873
5	3536215	2102125
6	3524864	2153641
Mean	3535639	2140318
Std. dev	20875.344	19024.101
% RSD	0.59	0.89

Table 5: Results of Intermediate Precision

Area of Cisplatin	Relative standard Deviation	Area of Topotecan	Relative standard Deviation
3562012	0.42	2102568	0.85
3521365		2145278	
3547215		2135624	
3535626		2135265	
3528692		2155869	
3548576		2143206	

Table 6: Results of Accuracy

Accuracy	Amount of Cisplatin	% Recovery	Amount of Topotecan	% Recovery
50	25	99.9	10	98.2
100	50	99.9	20	99.5
150	75	99.7	30	99.8

Table 7: Results of Robustness

Parameter	% RSD of Cisplatin	% RSD of Topotecan
Flow (0.8 mL/min)	0.57	0.38
Flow (1.2 mL/min)	0.64	0.87
Organic phase (36:64)	0.21	0.42
Organic phase (44:56)	0.69	0.56

Table 8: Results of Forced Degradation

Stress Parameter	% of Degradation	
	Cisplatin	Topotecan
Acid degradation (1N HCl)	14.6	14.9
Alkali degradation (1N NaOH)	14.1	15.3
Peroxide degradation (30% Peroxide)	15.2	16.6
Reduction degradation (30% sodium bisulphate)	13.9	12.4
Thermal (sample, 70°C, 6 Hrs)	13.4	11.5

range, six series of standard solutions were selected. Using peak area versus concentration of the standard solution, the calibration curve was plotted and the regression equations were measured. The least-squares method was used to calculate the slope, intercept and correlation coefficient.

Stress Degradation

Stress Degradation should not have any interference between the peaks of forced degradation preparations obtained for the chromatogram. Stress degradation studies were conducted in accordance with ICH guidelines Q1 (A) R2. The peaks of degradation should be well spaced and the resolution between the peaks should be at least 1.0. The peak purity of the principle peaks will pass only when there is separation. Forced degradation studies were performed to obtain the degradation of about 20 per cent by different types of stress conditions.

Robustness

An analytical procedure's robustness is a measure of its ability to remain unchanged by small but intentional differences in method parameters and gives an indication of its reliability during normal use. Robustness study was carried out by injecting a standard solution into the HPLC system and modifying chromatographic settings such as flow rate (±0.2 mL/min), organic content in the mobile phase (±10%). By establishing the effect of the modified

parameters, the separation factor, retention time and peak asymmetry were estimated.

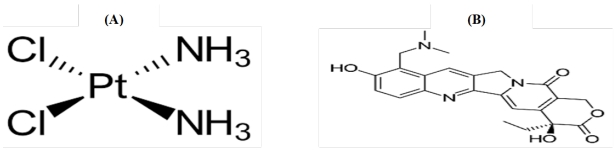


Figure 1: Structural representations of (A) Cisplatin and (B) Topotecan

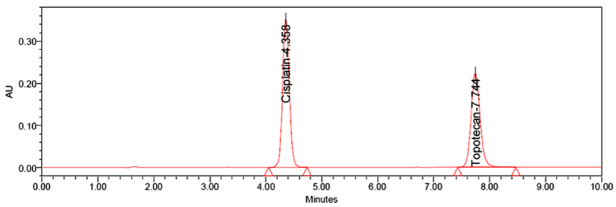


Figure 2: Chromatogram of System Suitability

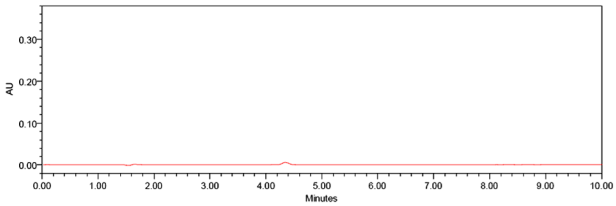
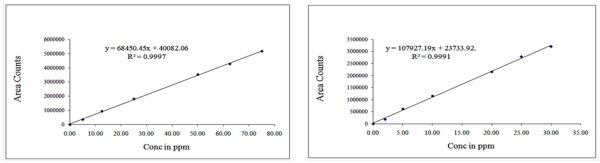


Figure 3: Chromatogram of Blank



A B

Figure 4: Calibration plots of (A) Cisplatin and (B) Topotecan

RESULTS AND DISCUSSION

The present study was designed to develop a simple, accurate and rapid analytical RP-HPLC method, that can be used for the simultaneous estimation of cisplatin and topotecan in bulk and pharmaceutical dosage form for the analysis of the assay method. In order to provide good assay performance, the chromatographic conditions were optimized. To optimize mobile phase, various combinations were tried for cisplatin and topotecan.

The final working mobile phase is (0.1%) triethylamine and acetonitrile in the composition of (60:40 v/v) Mobile phase for each drug were selected on the basis of its polarity. In order to obtain sufficient sensitivity for the two APIs in smaller proportions (cisplatin and topotecan) detection was carried out in several wavelengths.

Finally, the 262 nm wavelength was selected, where the two drugs showed good absorption. The flow rate was 1.0 mL/min. For Cisplatin and Topotecan, the retention time was 4.358 min, 7.744 min, respectively. The proposed method is validated by all the outcomes within limits in accordance with the ICH guidelines. The detection was carried out with a total runtime of 10.0 min. Below Table 1 represents the optimized chromatographic method.

System Suitability

The System Suitability was performed by injecting a standard solution containing 50 μg/mL of cisplatin and 20 μg/mL of topotecan in six replicates. The results indicate that the system suitability parameter is within the limit. Table 2 represents the system suitability results and Figure 2 represents the standard chromatogram.

Specificity

There was no interference from blank at the retention time of cisplatin and topotecan. Below Figure 3 represents the blank chromatogram.

Linearity

By plotting a calibration curve of the peak area against its respective concentration, linearity was determined. From this calibration curve, it was found that the curve was linear in the range of 5-75 μg/mL of cisplatin and 2-30 μg/mL of topotecan.

The regression equations for calibration curve of cisplatin was Y= 68450.45x + 40082.06 (R^2-0.9997) and Y= 68450.45 + 40082.06 (R^2) for topotecan respectively. Linearity results were shown in Table 3, calibration plots of cisplatin and topotecan were shown in Figure 4.

Precision

In terms of intraday and intermediate precision variations, the accuracy of this method was assessed. Six repeated analyses of the sample solution of cisplatin and topotecan on the same day under the same experimental conditions were used to determine the intraday studies.

In the same laboratory, the intermediate precision of the method was carried out by studying the analysis with different analysts and with different instruments. As percentage RSD values were found to be <2 per cent, the method is highly precise.

At each added concentration, good recoveries of the drug were obtained, indicating that the method was exact. Method precision results were shown in the below Table 4 and Table 5 represents intermediate precision results.

Intermediate Precision (Ruggedness)

Accuracy

By calculating the recovery experiments at three levels, the accuracy of the method was achieved (50%, 100% and 150%). APIs were prepared with a concentration of 25, 50, 75 μg/mL of cisplatin and 10, 20, 30 μg/mL of topotecan. For each spike level, the test solution was injected three times and the assay was performed as per the test method. The recovery outcomes were close to 100% and also the RSD values were less than $\pm$2% as well. The recovery percentage, mean and relative standard deviations have been calculated. Recovery values showed within the desired range. The method was accurate. Accuracy results were given in Table 6.

Robustness

Robustness of the chromatographic technique was determined by changing the flow rate and movable phase composition. % RSD was found to be within the acceptable limit. Robustness results were shown in Table 7.

Forced Degradation

The proposed method can be used for release and stability studies for effective evaluations and can be considered as stability preferable technique. The forced degradation study carried out according to the ICH requirements includes acid, base, oxidation, reduction, thermal degradation. From the chromatograms, it is evident that the selected drugs were stable under the applied stress conditions though degraded peaks were observed. Results of forced degradation were given in Table 8.

CONCLUSIONS

In this study, a novel, rapid, economical, sensitive and easily available HPLC method was evolved for the simultaneous estimation of cisplatin and topotecan in API and pharmaceutical dosage form. The main advantages of this method are no HPLC methods were reported. Other merits are shorter run time, low price, accessibility, sensitivity, reliability and reproducibility. These properties are important when a more number of samples are to be analyzed. The validation of all the parameters like linearity, accuracy, specificity, robustness, method precision were done and found to be within the acceptable limit. The relative standard deviation values for all the parameters were found to be less than 2%, which indicates the validity of the method and the results gained by this technique are in fair agreement. So the proposed method could be easily applied for the routine analysis and the pharmaceutical formulations of cisplatin and topotecan in quality control laboratories without any preliminary separation.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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